EXTENDED REPORT

Associations between the *PTPN22* 1858C→T polymorphism and radiographic joint destruction in patients with rheumatoid arthritis: results from a 10-year longitudinal study

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Objective: To investigate whether the *PTPN22* 1858T risk variant is associated with the rate of radiographic progression in rheumatoid arthritis (RA).
Methods: A longitudinally followed cohort of 238 Norwegian patients with RA (the EURIDISS cohort) was genotyped for the *PTPN22* 1858C→T polymorphism. Radiographic damage was assessed by hand radiographs at baseline and after 1, 2, 5 and 10 years, and the radiographs were scored with the Sharp method modified by van der Heijde (Sharp–van der Heijde score) by a single experienced reader. Baseline serum levels of rheumatoid factor and anti-cyclic citrullinated peptide autoantibodies were also examined.
Results: The reported association between RA susceptibility and carriage of the T allele (34.4% in patients vs 21.4% in controls; odds ratio 1.92, 95% confidence interval 1.36 to 2.71, p = 0.0002) was confirmed. An association between annual progression rate of Sharp–van der Heijde score and T-allele carriers (p = 0.01),was also found, which was also present when only patients positive for the shared epitope were analysed (p = 0.03). This association was also maintained in multivariate analyses adjusting for shared epitope and demographic variables.
Conclusions: An association between the *PTPN22* risk variant and increased progression rate for structural damage was found. The results indicate that the *PTPN22* gene may not only be associated with disease susceptibility, but also with disease progression.

Rheumatoid arthritis (RA) is a destructive inflammatory disease resulting in bone erosion and cartilage loss. The destruction is considered to be of an autoimmune nature, and autoantibodies are often detected in patients. Several risk factors, both genetic and environmental, seem to determine disease onset.[1] Specific alleles at the *HLA-DRB1* locus encoding the shared epitope (SE) are well-established contributors to RA susceptibility,[2] whereas only one non-human leucocyte antigen gene has convincingly been shown to be involved in RA predisposition. Carriage of a missense polymorphism (1858C→T; rs2476601) in the protein tyrosine phosphatase N22 (*PTPN22*) gene has, after the initial report,[3] repeatedly been shown in multiple independent studies to confer an increased risk of developing RA.[4–18] We have also previously reported an association with RA in a Norwegian population.[4] Overall, the studies have not detected an association between the SE and the *PTPN22* risk variant. The *PTPN22* polymorphism has also been found to influence the risk of other autoimmune diseases[19] such as systemic lupus erythematosus, Graves disease, myasthenia gravis, and in particular, type 1 diabetes, in which the association was first reported.[20] However, some autoimmune diseases, eg multiple sclerosis, inflammatory bowel disease, psoriasis and coeliac disease do seem to be associated.[19] Notably, *PTPN22* seems to be associated with the group of autoimmune diseases that are classically characterised by circulating autoantibodies.

PTPN22 encodes lymphoid tyrosine phosphatase, which modulates the activation of kinases such as Lck, involved in early events of T cell-receptor signalling. The predisposing 1858T allele encodes a protein with higher catalytic activity, which is a more potent negative regulator of T cell activation.[21] The autoimmune risk variant is probably a gain-of-function allele. A theory to explain the seemingly unintuitive association between autoimmune diseases and suppressed T cell signalling is that it could cause either insufficient activity of regulatory T cells or a failure to destroy autoreactive T cells in the thymus.[21]

Genetic risk factors may also influence the disease phenotype and the clinical outcome. The SE has been found to play a role in RA progression, leading to more severe forms of disease.[22 23] The potential influence of the *PTPN22* polymorphism on disease progression is less clear.[6 7 13 14 18] The focus with regard to disease phenotype has mostly been on autoantibodies, and studies examining the association between *PTPN22* and rheumatoid factor (RF) or anti-cyclic citrullinated peptide (anti-CCP) have given conflicting results.[3 5–9 12 15 24] The SE is known to be associated with the presence of both RF and anti-CCP.[25] Furthermore, both RF and anti-CCP autoantibodies have been shown to be associated with progression of structural damage in RA.[26–28]

We have previously reported 10-year follow-up data of the Norwegian European Research on Incapacitating Disease and Social Support (EURIDISS) cohort.[29] The main objective of the present study was to use the data from this cohort to investigate whether the *PTPN22* polymorphism predicts the rate of radiographic progression in RA. In addition, we studied the association between the *PTPN22* and the SE.

Abbreviations: ACR, American College of Rheumatology; ANOVA, analysis of variance; CCP, cyclic citrullinated peptide; EURIDISS, European Research on Incapacitating Disease and Social Support; ESR, erythrocyte sedimentation rate; HAQ, Health Assessment Questionnaire; JSN, joint-space narrowing; *PTPN22*, protein tyrosine phosphatase N22; RA, rheumatoid arthritis; RF, rheumatoid factor; SE, shared epitope

METHODS

Patients

Our available Norwegian EURIDISS cohort, which has been longitudinally followed up, was used in this explorative and hypothesis-generating study. The 238 patients (175 female, 63 male) fulfilled American College of Rheumatology (ACR) criteria for RA.[30] The included patients had a mean age of 52.0 (range 20 to 70) years and a disease duration of <4 years (mean 2.3) at baseline.

Blood samples were taken at baseline, and serum was frozen at −70℃ for later analysis. The presence of IgM-RF and IgA-RF was analysed using ELISA. A commercial ELISA kit assay (INOVA Diagnostics Inc., San Diego, CA, USA) was used to measure anti-CCP. Antibody concentrations were 1– 251 U/ml for anti-CCP and 1–301 U/ml for IgM-RF and IgA-RF. Baseline serum levels were used to group the patients, and the cut-off for positivity for IgM-RF, IgA-RF or anti-CCP was defined as titres >25 U/ml.

The following variables were used to describe the disease at baseline and during the follow-up assessments at 1, 2, 5 and 10 years: erythrocyte sedimentation rate (ESR) as a marker for disease activity, Health Assessment Questionnaire (HAQ) score[31] as a measure of physical disability, and grip strength as a performance-based measure for muscle strength and physical function. Grip strength was measured with a hand-held dynamometer (Jamar; Smith and Nephew, Irwington, NY, USA), and average grip strength was calculated based on the best performance of two attempts on each hand.[32 33]

The same measures of radiographic damage scores were used during each of the five examinations during the 10-year follow-up period. Only patients with radiographic measurements of the hands at both baseline and the 10-year examination were included in the progression analysis (n = 144). The method for the radiographic assessments has been described in detail elsewhere.[29] In brief, the radiographs were scored by the Sharp score modified by van der Heijde (Sharp–van der Heijde score) by a single experienced reader[34 35] with known time order. In total, 16 areas for erosions (score 0–5) and 15 areas for joint-space narrowing (JSN; score 0–4) in each hand yield a potential maximum total score for both hands of 280, a maximum erosion score of 140 and JSN score of 120. Joints that could not be read (not visible on the radiographs or those in patients who had undergone joint replacements/arthrodesis surgery) were given the last available score (last observation carried forward).

Genotyping

DNA was available for 221 of the patients with RA. Whole-genome amplification of the DNA was performed before genotyping (GenomiPhi Amplification Kit; GE Healthcare, Little Chalfont, Buckinghamshire, UK). This is a reliable method, yielding high-molecular amplified DNA thoroughly validated for genotyping purposes.[36]

The *PTPN22* 1858C→T polymorphism was genotyped by allelic discrimination using a Taqman polymerase assay (Applied Biosystems, Foster City, CA, USA), as previously described,[4] followed by detection on a real-time PCR system (ABI7000; Applied Biosystems). To assess whether the 1858C→T polymorphism was associated with RA in this cohort, we compared the *PTPN22* genotyping data from the patients with RA with our previously published genotyping data from 555 controls.[4] Both patients and controls were in Hardy–Weinberg equilibrium, and the genotyping success rate was >95%.

Patients with RA were genotyped for *DRB1* by sequencing[37] (BigDye Terminator Kit V.3.1; Applied Biosystems) on a DNA analyser (ABI3730; Applied Biosystems) followed by allele assignment (AssignSBT V.3.2.7; Conexio Genomics, Applecross, Australia).[38] In total, 95% of the patients were successfully *DRB1* genotyped. Individuals who carried at least one copy of the *DRB1* alleles (0101, 0102, 0401, 0404, 0405, 0408, 1001 and 1402) were classified as SE-positive.

Statistical analysis

The Pearson χ^2 test or Fisher exact test as appropriate were used for categorical variables, including the genotype analyses. Odds ratios (ORs) and 95% confidence intervals (CI) were calculated according to the Woolf–Haldane method. Mean values with standard deviation (SD) were calculated for normally distributed continuous variables, and group comparisons were performed with two-tailed, two-sample *t* tests. The distribution of radiographic scores and progression rates were skewed. Scores were therefore described by median and interquartile range. The Mann–Whitney *U* test was used to compare progression rates across the genetic markers. Multivariate regression analyses were performed to examine the independent effect of *PTPN22* on annual radiographic progression rates, controlling for the SE and demographic variables. Mixed-models, repeated-measures analysis of variance (ANOVA) was performed to assess the effect of *PTPN22* on the change in radiographic rates, controlling for age, gender and disease duration. Jacknife residuals and Cook *D* statistic were used to assess the underlying assumptions of the regression model(s). For the mixed models assessment, the Cook *D*, the covariance ratio statistic and the covariance trace statistic were used.

Generally, no adjustment for multiple testing was carried out as this was an explorative and hypothesis-generating study. Only p values in the test of the main hypothesis investigating the influence of *PTPN22* on the annual progression rate (table 1) were corrected using the Simes procedure.[39] In accordance with this procedure, because six tests were performed, a correction factor of 2.45 was applied. Statistical analyses were performed using SPSS V.11.0 (SPSS Inc., IL, USA) and SAS (SAS Institute Inc., Cary, NC, USA.) software programs for the mixed-models repeated-measures analysis. Significance was set at $p<0.05$.

RESULTS

We confirmed the reported RA association with carriage of the T allele at position 1858 of *PTPN22* (OR = 1.92, 95% CI 1.36 to 2.71, $p<0.001$) (table 1). No sign of the proposed dose effect[12 19] was evident, as the genotypic distributions of T/T, C/T and C/C were as follows: 0.9%, 33.0% and 66.1% among cases vs 1.6%, 19.8% and 78.6% among controls, respectively. Noticeably, there was no trend towards an increased frequency of 1858T homozygotes among cases compared with controls.

All patients, both autoantibody-positive and autoantibody-negative, showed a higher frequency of the presence of the 1858T allele compared with controls (table 1), although this did not reach significance in the anti-CCP-negative group. In general, more 1858T carriers were seen among the group of

Table 1 Distribution (%) of *PTPN22* 1858T allele carriers among controls and patients with RA

	1858T carriers	OR (95% CI)	p Value
Controls (n = 555)	21.4		
Patients			
All (n = 221)	34.4	1.92 (1.36 to 2.71)	0.0002
RF+ (n = 101)	39.6	2.41 (1.54 to 3.75)	0.0001
RF− (n = 114)	29.8	1.57 (1.00 to 2.44)	0.05
Anti-CCP+ (n = 129)	38.0	2.25 (1.50 to 3.37)	0.00009
Anti-CCP− (n = 86)	29.1	1.51 (0.92 to 2.49)	0.1

CCP, cyclic citrullinated peptide; PTPN22, protein tyrosine phosphatase N22; RA, rheumatoid arthritis; RF, rheumatoid factor.

Table 2 Demographic and disease characteristics at baseline for carriers and non-carriers of the *PTPN22* 1858T allele

	Carriers (n = 74)	Non-carriers (n = 141)	p Value*
Age (years), mean (SD)	50.7 (13.0)	51.9 (13.0)	0.50
Female (%)	80	72	0.25
Disease duration (years), mean (SD)	2.4 (1.2)	2.1 (1.2)	0.08
SE+ (%)	76	76	1.0
Antibody titres, mean (SD)			
Anti-CCP (U/ml)	114 (104)	95 (102)	0.21
IgM-RF (U/ml)	87 (102)	64 (89)	0.09
IgA-RF (U/ml)	48 (67)	47 (84)	0.96
Antibody-positive patients (%)			
Anti-CCP	66.2	56.7	0.19
IgM-RF	54.1	43.3	0.15
IgA RF	43.2	35.5	0.31
HAQ score (0–3), mean (SD)	0.94 (0.61)	0.93 (0.66)	0.90
CRP (mg/l), mean (SD)	11.6 (13.4)	12.8 (18.7)	0.65
ESR (mm/h), mean (SD)	28.1 (18.5)	25.2 (21.0)	0.32
Grip strength (kg), mean (SD)	17.2 (11.3)	20.0 (11.8)	0.09
Radiographic variables (median, IQR)			
Sharp–van der Heijde score	4.0 (12 to 69)	0 (6 to 46)	0.002†
Erosion score	0 (3 to 21)	0 (2 to 10)	0.10
JSN score	4.0 (8 to 49)	0 (4 to 40)	0.001

CCP, cyclic citrullinated peptide; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; HAQ, Health Assessment Questionnaire score; IQR, interquartile range; JSN, joint-space narrowing; *PTPN22*, protein tyrosine phosphatase N22; RF, rheumatoid factor..
*χ^2 test applied to categorical variables, two-sample *t* test applied to continuous variables, Mann–Whitney U test applied to radiographic variables.
†Radiographs available from 103 vs 52 patients.

autoantibody-positive patients, but no significant heterogeneity was observed compared with the autoantibody-negative patients when grouped by RF (39.6% in the RF+group vs 29.8% in the RF− group, p = 0.13) or anti-CCP (38.0% in the anti-CCP+ group vs 29.1% in the anti-CCP− group, p = 0.18) status. The percentage of autoantibody-positive patients and their mean titres were not significantly higher in *PTPN22* 1858T carriers than in non-carriers (table 2).

The demographic and clinical baseline characteristics are shown in table 2. Only the scores for Sharp–van der Heijde and JSN were significantly higher in 1858T carriers than in non-carriers at baseline.

The annual progression rate of structural damage was significantly increased in carriers compared with non-carriers of *PTPN22* 1858T, and this difference was consistent across all three radiographic endpoints (annual Sharp–van der Heijde score, annual erosion score and annual JSN score; table 3). The differences in annual Sharp–van der Heijde score and annual erosion score were also significant after correction for multiple testing (p = 0.02 and p = 0.05, respectively). The radiographic scores were higher at baseline (mean disease duration 2.3 years) in 1858T carriers compared with non-carriers, and this difference increased over time (fig 1). Changes in HAQ scores and other measures of health status and markers of inflammatory activity were not significantly different between 1858T carriers and non-carriers (data not shown).

Table 3 Annual progression of radiographic damage for carriers and non-carriers of the *PTPN22* 1858T among all patients and among patients positive for the SE

	Carriers	Non-carriers	p Value†
All patients‡			
Sharp–van der Heijde score	3.4 (5.9 to 12.0)	1.4 (4.0 to 11.1)	0.01
Erosion score	1.0 (2.1 to 5.2)	0.4 (1.2 to 5.2)	0.02
JSN score	2.1 (3.8 to 6.8)	1.0 (2.9 to 6.3)	0.03
SE-positive patients§			
Sharp–van der Heijde score	4.0 (5.2 to 12.0)	2.6 (4.5,11.1)	0.03
Erosion score	1.2 (2.2 to 5.2)	0.7 (1.5 to 5.2)	0.02
JSN score	2.4 (3.3 to 6.8)	1.5 (2.7 to 6.3)	0.08

IQR, interquartile range; JSN, joint-space narrowing; *PTPN22*, protein tyrosine phosphatase N22; SE, shared epitope.
*Values are median (interquartile range).
†Mann-Whitney U test was applied to radiographic variables.
‡Carriers (n = 48) and non-carriers (n = 96).
§Carriers (n = 38) and non-carriers (n = 69).

Carriers of 1858T were as frequent in SE-positive (n = 158) as in SE-negative (n = 49) patients (34.7% vs 34.8%, p = 0.99). The difference in radiographic progression in *PTPN22* 1858T carriers compared with non-carriers was also present when only patients positive for the SE (table 3, fig 2) were investigated, even though the difference for JSN did not reach significance. However, the difference in erosion score remained significant after correction for multiple testing (p = 0.05). The annual progression scores were higher among patients positive for both *PTPN22* 1858T and the SE than for those only positive for the SE (table 3).

We chose to use non-parametric tests for the comparative analyses of the radiographic data due to skewed distribution. However, we also performed group comparisons with two-sample t tests and obtained similar results.

We performed multivariate regression analysis to further explore whether *PTPN22* 1858T predicts radiographic progression independently of the SE, and included the following variables: age, disease duration, gender, *PTPN22* 1858T status and SE status. Interaction between *PTPN22* 1858T and the SE was excluded and residuals were checked for normality. The multivariate analysis confirmed the independent association between *PTPN22* and the annual radiographic progression rates of the Sharp–van der Heijde score and the erosion score, and the association for the annual progression rates of JSN almost reached significance (table 4). Bivariate regression analyses showed that the explained variance R^2 was 0.05 for *PTPN22* 1858T carriers and 0.07 for presence of the SE.

The effects of *PTPN22* 1858T on radiographic progression scores were also significant in the mixed-model repeated-measures ANOVA adjusted for age, gender and disease duration (p<0.009). The adjusted least square mean values are shown in figures 1 and 2.

DISCUSSION

It is established that *PTPN22* 1858C→T is associated with RA disease susceptibility.[3–14] Our current results in this 10-year follow-up study indicate that presence of *PTPN22* 1858C→T also predicts radiographic progression. The external validity of radiographic progression has been questioned because an association between structural damage and physical disability had until recently only been shown in established disease.[40 41] In our study of patients with disease of short duration, we recently showed a longitudinal association between radiographic damage and reduced physical functioning.[29] However, we were unable to show that the *PTPN22* risk variant was associated with progression in disability level, which is not surprising in view of the low annual progression rate in physical functioning in this cohort.[29] Furthermore, carriage of the *PTPN22* 1858T allele seems to be a weaker predictor of radiographic progression than the SE, acute-phase reactants, anti-CCP and RF.[29 42–45]

Some studies have also previously indicated that *PTPN22* might influence disease course. A non-significant trend towards higher Larsen scores over time in patients positive for

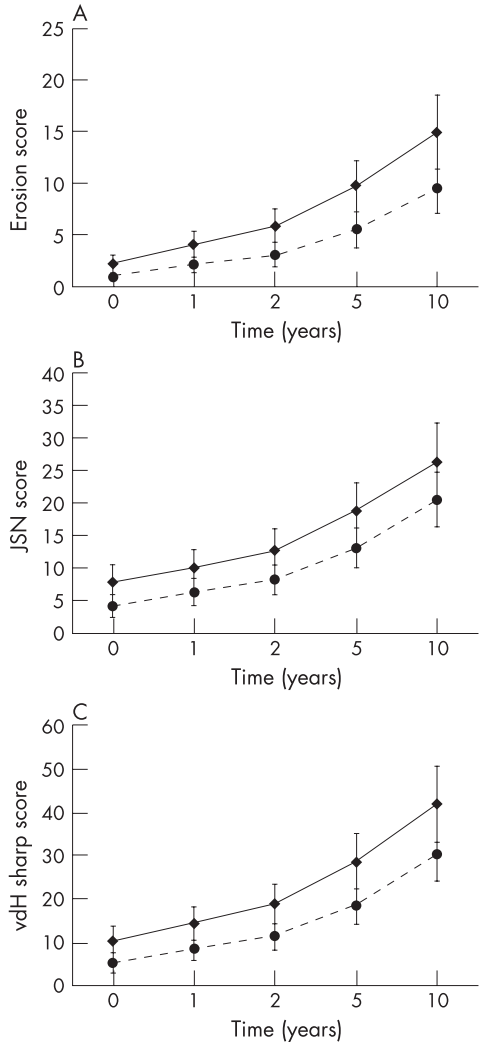

Figure 1 Radiographic progression in patients with RA divided into *PTPN22* 1858T positive (n = 48; solid line) and negative (n = 96; broken line) at different time points during the 10-year follow-up period. (A) Erosion scores; (B) joint-space narrowing scores; (C) van der Heijde-modified Sharp score. Values are least squares means (95% CI) from mixed-models repeated-measures analysis of variance adjusted for age, gender and disease duration.

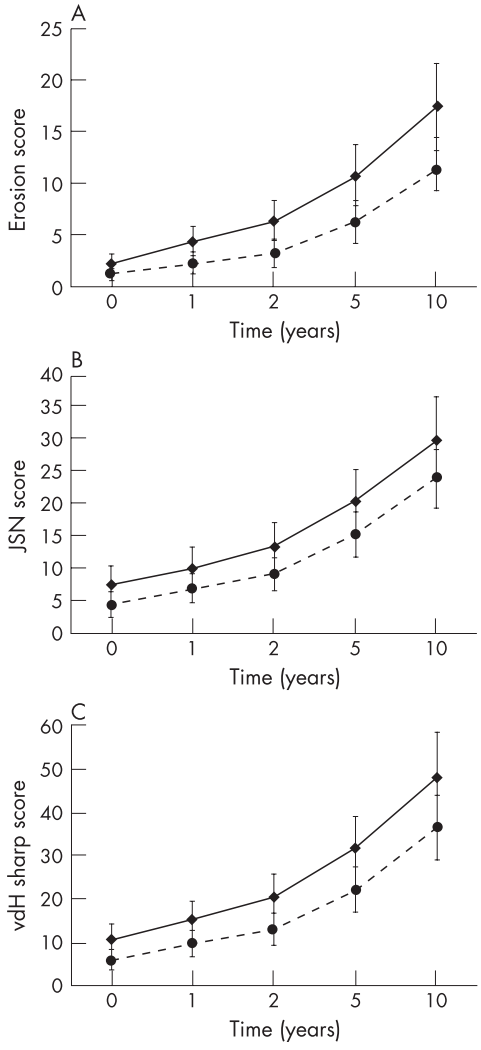

Figure 2 Radiographic progression in SE-positive patients with RA divided into *PTPN22* 1858T positive (n = 38; solid line) and negative (n = 69; broken line) during the 10-year follow-up period. (A) Erosion scores; (B) joint-space narrowing scores; (C) van der Heijde-modified Sharp score. Values are least squares means (95% CI) from mixed-models repeated-measures analysis of variance adjusted for age, gender and disease duration.

the *PTPN22* 1858T allele, but not at study entry, has been observed.[13] Furthermore, by dividing patients into those with and without erosive disease, the frequency of the T allele has been found to be higher, although not significantly so, in patients with erosive disease.[6] In a third study, no association between the *PTPN22* risk allele and the rate of joint destruction (Sharp–van der Heijde score) was observed.[18] The discrepancy between the latter and our study could be explained by their shorter follow-up time of only 4 years, and in addition, the *PTPN22* risk effect was generally less pronounced in their cohort (OR = 1.37[46]). Overall, the reported ORs range from 1.37 to 2.04,[47] and in our cohort we observed an OR in the upper part of this range (table 1). Interestingly, in type 1 diabetes, the *PTPN22* genotype was recently reported to be associated with disease progression from pre-diabetes to clinical diabetes.[48]

As we had no a priori information about the effect size of the *PTPN22* risk variant on radiographic progression and were also constrained by the available longitudinal EURIDISS cohort, we considered this study to be only a hypothesis-generating study. However, a post hoc power calculation based on our results suggests that 460 patients should be included in a follow-up study to obtain a power of 0.80 to repeat our findings. In spite of the limited number of patients in the current study, the results were surprisingly robust, with consistent findings across three radiographic endpoints, findings maintained in the subgroup of SE-positive patients, and also significance maintained after correction for multiple testing and in multivariate analyses adjusting for demographic variables and presence of the SE.

Our data do not provide statistical evidence that the *PTPN22* 1858T allele has an effect on presence of autoantibodies. Overall, conflicting data has emerged regarding this relationship; studies have reported a possible association with RF-positive disease,[3 6 12 15] RF-negative patients [5 8 9] and patients with anti-CCP autoantobodies,[24] and that presence of autoantibodies is not influenced by the *PTPN22* 1858T allele.[7] Although the inconsistent results could partly be explained by clinical heterogeneity or differences in assessment of the autoantibody status (due to detection methods or disease duration), taken together, a clear correlation between RF status and *PTPN22* carriers is not apparent. However, presence of the SE in our cohort was clearly associated with both presence and increased levels of RF and anti-CCP (data not shown).

Table 4 Multivariate regression analyses using annual progression rates for Sharp–van der Heijde score, erosion score and JSN score as dependent variables

	Annual progression rate					
	Sharp–van der Heijde score		Erosion score		JSN score	
	B	p Value	B	p Value	B	p Value
Age	−0.016	0.40	−0.002	0.49	−0.013	0.27
Disease duration	0.45	0.03	0.14	0.09	0.30	0.02
Female sex	0.90	0.11	0.29	0.19	0.59	0.11
PTPN22 1858T	1.04	0.04	0.45	0.03	0.56	0.09
SE	1.89	0.001	0.67	0.004	1.27	0.001
R^2	0.13		0.10		0.13	

B, non-standardised β, JSN, joint-space narrowing; *PTPN22*, protein tyrosine phosphatase N22; SE, shared epitope.

Several studies have shown that the SE is associated with progression of radiographic damage, and we also found a clear association with increased progression in the SE-positive patients in this study (data not shown). However, no association between presence of the SE and *PTPN22* 1858T was observed, which has also repeatedly been shown by others, and indicates that the two risk factors act independently. More interestingly, we found, in a separate analysis, that patients carrying both the SE and the *PTPN22* risk variant had a faster progression rate (measured by the Sharp–van der Heijde score, as well as the individual JSN and erosion scores) than patients with the SE but without the *PTPN22* variant (table 3, fig 2). An independent effect of *PTPN22* was also supported by the results of the multivariate regression analyses (table 4). A similar, but not significant, tendency has been reported earlier using the Ratingen score for radiographic damage.[49]

Our data indicate that the *PTPN22* 1858T-allele might also play an independent role in disease progression, in addition to a role in disease susceptibility, in patients with RA. These results need confirmation, preferentially by further studies in larger cohorts with long-term follow-up. Such studies are also needed to clarify whether the *PTPN22* 1858T allele can be considered as a clinically relevant marker of progressive disease.

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